Resistance of the prothallia of Camptosorus rhizophyllus to desiccation *

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Camptosorus rhizophyllus (L.) Link is found growing with mosses and lichens on the shaded surface of dry limestone ledges and on detached limestone slabs in open ravines and torrent beds. Only rarely have groups been found in well-shaded or continually moist places in this region (southern Indiana). Growing in places thus exposed without constant water supply, the plants are subjected to brief periods of abundant moisture (during and immediately after precipitation) which alternate with longer periods of drought. That plants with a delicate prothallial stage in their life history could secure and retain residence under such conditions has been a cause for surprise.

The drought-resisting power of some greenhouse cultures of this fern grown in the spring of 1912 suggested a possible adaptation to its well-known xerophytic habitat. In an attempt to determine to what extent this ability to withstand drought might be a factor in adaptation, fronds with mature spores were collected in October, 1912, and cultures were made as usual on sterilized soil in clay saucers. These cultures were subjected to a variety of conditions to be later enumerated.

An attempt was made to obtain information on the following points: the uniformity of spore germination and prothallial development, the ability of prothallia to resist or survive natural drought conditions, and the ability to survive conditions leading to complete desiccation.

Fronds were collected on October 26, 1912, and kept between sheets of filter paper in a book in the laboratory. The sporangia were lightly crushed to free the spores and then sown on thoroughly sterilized soil, November 22, 1912. The cultures were kept in the greenhouse and were protected by bell-jars supported on

* The writer has been unable to find any literature bearing upon this subject, for the prothallia of this or any other homosporous fern.

small blocks of wood to provide adequate ventilation. Some cultures were exposed to direct sunlight while others were exposed to strong diffused light only. The temperature conditions were the same, 20–25° C. The soil was kept moist, not wet, but was allowed to show a dry surface for a period of 12 to 24 hours once each week. The first green was noticed December 17, at which time the prothallia when examined with a microscope were found to be composed of two to ten cells each. These cultures showed good growth, seeming to suffer no injury from the dry periods. Those in the sunlight showed rather a more rapid development than those in diffused light. On February 11, 1913, the culture in sunlight contained prothallia in good condition but varying in size from merely germinated spores up to plants 2–3 mm. broad with but very few antheridia or archegonia. The culture in diffused light did not show similar prothallia until March 21, 1913.

The spores of *Camptosorus rhizophyllus* germinate very irregularly. Twelve weeks after the spores were sown, a small bit of soil—not over 3 mm. square—removed from a culture where the plants seemed most thrifty, showed all stages in development from spores with the perinium just ruptured to prothallia bearing mature antheridia and archegonia. Many spores retain their vitality up to May in the dry atmosphere of the laboratory, and fronds collected in March furnished viable spores for cultures. The long dormant period of spores on the moist soil of cultures suggests that they might remain so on the soil of their habitat through the winter season.

Four methods of reducing water content were used. First, a glycerin desiccator was used, consisting of two glass vials through which, by means of an aspirator, a current of air was drawn after passing through two U-tubes containing glycerin and crumpled filter paper. Heaviest c.p. glycerin was used. The aspirator was arranged so that the air of the vials was changed about one hundred times each twenty-four hours. Second, cultures were left under bell-jars, exposed to the warm air and full sunlight of the greenhouse. Third, cultures were left under bell-jars, exposed to the dry air and diffused light of the greenhouse lobby or vestibule at a slightly lower temperature, 16–20° C. This place represents as nearly as possible the natural growth

conditions. In the second and third cases full ventilation was secured by allowing the jars to rest on blocks of wood 2 cm. high. Fourth, large portions of a culture were placed in a desiccator over c.p. sulphuric acid and the whole apparatus was kept in a cool, moderately lighted location. In all cases, when soil and prothallia were removed from a dry chamber they were placed in contact with moist soil under a bell-jar subject to full diffused light at a temperature of 16–20° C., for recovery. Examination for dead prothallia was made after three or four days under such conditions. Extreme care was taken at all times when removing portions of cultures to or from the dry chambers, to leave the prothallia as far as possible undisturbed and uninjured.

Results—glycerin desiccator.—A large portion of the soil of a culture was removed to the vial of the desiccator and allowed to remain in the dry air undisturbed, except as small portions were removed for recovery and growth. Specimens placed in the desiccator on March 22 seemed to revive completely up to April 22, at which time a few small dead prothallia were found in a portion removed. Another portion removed April 29 showed about 50 per cent of the prothallia dead. The last of the soil and prothallia was removed May 5. All the smaller plants and all but a very few of the larger plants were dead at that time.

In this set of experiments a very few fully matured prothallia survived continuous exposure to dry air for forty-four days. Very few were damaged by such exposure for a period of thirty days. That the recovery was complete in case of survival is proven by the continued growth of the prothallia and their later production of sporophytes.

That vigorous desiccation follows immediately after the plants were placed in the vials is shown by the fact that the soil had given up all free moisture in twenty-four hours after being placed therein. As a check, at the beginning of this experiment a clump of thrifty mature prothallia of *Onoclea Struthiopteris* was placed in one of the vials. After forty-eight hours' exposure to the dry air not one plant recovered.

A similar set of experiments was arranged with the whole apparatus exposed to the direct sunlight. Most of the prothallia so exposed were dead at the end of twenty-eight days, and all were dead after thirty-five days of exposure.

Results—sulphuric acid desiccator.—A portion of soil bearing prothallia was removed to a porcelain dish in a desiccator containing c.p. sulphuric acid. The lid was sealed down with vaseline and the apparatus placed in diffused light at a temperature of 16–20° C. After eighteen hours the prothallia showed a marked yellowish color. A portion removed after four days showed a recovery of but two or three per cent. The plants removed at this time recovered very slowly, requiring a week to resume a normal appearance.

Results—normal dry air.—The soil mass of a culture was divided into two approximately equal parts and carefully removed to sterilized clay saucers. One portion was placed under a bell-jar exposed to the full sunlight in the greenhouse. The other was kept under a bell-jar exposed to full diffused light and at a temperature of 16–20° C., an average of four degrees lower than that for the first portion. After three weeks, but a few of the plants exposed to direct sunlight recovered, and all were dead after five weeks of such exposure. Of the second lot not exposed to direct sunlight a portion removed after thirty-four days showed almost complete recovery. After fifty-five days, about 25 per cent of the prothallia recovered. After sixty-three days, a very few of the largest plants recovered and continued to grow.

The conditions under which the second group of plants was kept approximate very closely the summer conditions in the regular habitat of *Camptosorus rhizophyllus*. The results of that set of experiments certainly suggest an explanation of this plant's abundant growth under such conditions. It should be noted here, as above, that full recovery of the plants has been demonstrated by their continued growth and later production of sporophytes. If mature prothallia can withstand continuous drought for two months, they would certainly survive the difficulties of the average season after late March, at which time spores may germinate outside. The occasional rains through the summer would make possible recovery, fertilization, and the production of sporophytes.

In connection with this set of experiments another should be noticed. A culture was prepared as for all the above on November 25, 1912. The drainage was such that a part of the soil

surface was dry at all times, except when occasionally flooded. The remaining portion was moist as in the other cultures. All gradations of moisture were in evidence in and between the two regions. Small prothallia were seen on December 18 on the damp soil. On March 1, 1913, most of the prothallia of this region showed mature sex organs. The drier portions at the latter date showed germinating spores, dwarf male prothallia, and later stages in development up to mature plants. After March 1, this culture was watered irregularly and was allowed to become quite dry each time before more water was applied. After April 15 the culture was screened from the direct sun. Water was occasionally applied so as to flood the whole surface. On May 5 there were many living prothallia and several young sporophytes. On June 9 there were several large, living prothallia and the young sporophytes were uninjured, although during the previous month the culture had been four times dry, once for seventy-two hours.

SUMMARY

As has been stated above, the experiment of subjecting prothallia to normal dry air without direct sunlight,—continuous conditions approximating the average of varying conditions found in nature,—has shown that the production of mature prothallia under such conditions is possible. The other experiments of subjecting prothallia to more thorough desiccation in the glycerin desiccator and over sulphuric acid show the possibility of surviving the extreme conditions found in nature. There can remain but little doubt that the drought-resisting character of the prothallia is a very effective factor in the adaptation of *Camptosorus rhizophyllus* to its habitat.

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